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Original Article

Investigation of the Pro-active Role of Alpha Amyrin Nanoemulsions in Quashing Neurodegeneration, Excitotoxicity, and Neuronal Inflammation-A Combined *in vivo* and *in silico* Approach

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ABSTRACT

Background: Aluminium is a pervading metal that poses a serious threat causing severe brain damage and neuro-degeneration. Alpha amyrin a pentacyclic triterpene despite its potency has not been properly utilized to treat neurodegenerative disorders because of its erratic GI absorption and poor BBB permeability. **Purpose:** To determine the role of alpha amyrin nano-emulsion in neuroinflammation induced by aluminium through an *in vivo* and *in silico* approach. **Materials and Methods:** An *in silico* approach was opted to determine the possible interactions of the drug with various inflammatory markers and was performed using Autodock. A chitosan-decorated nano-emulsion of alpha amyrin was prepared, characterized, and administered intranasally to Wistar albino rats for 42 days. The role of the treatment on neuroinflammation was measured by checking levels of *SOD, catalase*, inflammatory markers like IL-6, TNF-α, and acetylcholine esterase, and glutamate levels in the brain. Also, histopathology studies were performed in detail. **Results:** The results confirm that alpha amyrin interacts well with the inflammatory markers with a good docking score, thereby inhibiting them. The administration of nano-emulsion brings about *improvement in the anti-oxidant status of* the *brain*, reduction in neuroinflammatory markers, and acetylcholine esterase levels in rat brains. Histopathology of rat brains shows that alpha amyrin nano-emulsion administration brought about neuro-protection as it increased the number of intact neurons, and lowered neuronal damage, gliosis and pyknosis brought about by aluminium. **Conclusion:** The results and findings confirm the pro-active role of alpha amyrin nano-emulsion in quashing neuro-degeneration and neuro-inflammation in aluminum-induced neuro-toxicity.

Keywords: Aluminium, Alpha amyrin, Neuro-toxicity, Inflammatory markers, Nano-emulsion, Docking, Neuro-protection.

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INTRODUCTION

Neuro-toxicity, excitotoxicity, and neuronal inflammation are interchangeable terms as all of these result in neuro-degeneration and brain damage and involve overload of calcium, oxidative stress induced by free radicals, lipid peroxidation, and neuronal loss. Aluminium is a hazardous metal to which we get exposed on a daily basis. Exposure to aluminum over a period of time leads to severe degenerative changes in our brain resulting from oxidative stress which contributes to neuronal inflammation neuronal loss, cognitive impairment and conditions like dementia, Alzheimer's, etc.[1] Neuroinflammation alters the density of the dendritic spine, which, in turn, influence cognitive function decreases the density of the dendritic spine and impairs learning and memory in developing rats.[2] Neuroinflammation is associated with the pathogenesis of learning and memory deficits. The hippocampus with abundantly expressed receptors for proinflammatory cytokines such as Interleukin-1β (IL-1b), Interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) is especially vulnerable to injury and inflammation. Pro-inflammatory cytokines can impair Long-Term Potentiation (LTP), and inhibit neurotrophins which are important for neuronal survival/function.[3]

Oxidative stress is a frittering process that contributes to lipid peroxidation, cellular aging, degeneration, and apoptosis. The brain is a very sensitive organ that is mainly comprised of lipids

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and is highly prone to aluminium chloride-induced oxidative stress which deranges the antioxidant status of the central nervous system. This negatively impacts the histoarchitecture of the brain causing neuro-degeneration, neuronal inflammation, and neuronal apoptosis.[4] Aluminium has easy access to the brain as it gets carried across the Blood-Brain Barrier (BBB) by binding to the transferrin receptors and then commingling and fusing with the brain.[5] Neuroinflammation is a key factor contributing to neurodegenerative conditions, and microglial activation plays a major role in the inflammatory process. This mainly contributes to the neurobehavioral, neurochemical, and cognitive deficits.[6]

Microglia and the astrocytes, the inherent cells of immunity in the brain enact a pivotal role in initiating neuronal inflammation which is concorded by the damages instigated by inflammatory mediators activated by them like chemokines, cytokines, and other reactive species. Microglia exhibits a stunted turnover rate which puts it at risk of degenerative changes inflicted by pro-inflammatory mediators. Augmented stimulation of the microglial triggers the release of neuroinflammatory cytokines namely IL-1β, TNFα, and IL-6 that contributes to morbid alterations embracing neurocognitive and behavioral derangements.[7] Microgliosis leads to a pro-inflammatory microglial phenotype with a decline in phagocytic and tissue conservatory functions. Memory and learning process demands augmented neuroinflammatory gestures between cells of immunity and the neuronal system.[8]

Aluminium inflicts deleterious effects predominantly in the cerebral cortex and the hippocampus, the areas of learning and memory in the brain[9] contributing to the accretion of amyloid β and hyperphosphorylation of tau, resulting in neurofibrillary tangles and plaques formation. It deranges cholinergic neurotransmission by increasing the enzymatic activity of acetylcholine esterase, induces lipid-protein damage and destabilizes endogenous antioxidant enzymes and Na+/K+ATPase activity, and causes overexpression of Cyclin-dependent kinase5 (Cdk5).[10] Also, aluminium exposure leads to degenerative changes altering the histoarchitecture of brain, histopathological changes neuronal loss, and cellular abberations.[11] Aluminium intoxication and would derange the antioxidant status, neurotransmitter levels and thereby contributes to oxidative stress-induced damage that imitates Alzheimer's. Neurobehavioral and cognitive aberrations and inflammatory processes are involved along with lesions in brain areas such as the cortex and hippocampus.[12]

Aluminium exposure over a long period causes activation of microglia which triggers the release of oxidative stress inducers like NO, OH, H_2O_2, O_2 radical, etc. Aluminium deranges NO synthase and guanylate cyclase activation by glutamate. nNOS, eNOS and iNOS are the isoforms of NOS in the brain transgressed due to Aluminium inebriation contributing to immoderate levels of NO. Inappropriate levels of NO result in neuro-degeneration resulting from oxidative stress and other cellular mechanisms as NO combines with O_2 formed due to the Fenton reaction interacting with NO to produce peroxy nitrate a harmful byproduct.[13] Nitric oxide produced by nitric oxide synthase plays a role in regulating blood flow to the brain and dendritic spine growth. But nitric oxide sourced from inducible nitric oxide synthase seen in vascular endothelium may act as a neurotoxicant. Microvessels of Alzheimer's brain high nitric oxide synthase activity. In the case of Alzheimer's, high levels of inducible NOS may lead to switching over of messenger activity of nitric oxide to neuro-toxicity-inducing mode.[14] Inhibition of NO synthesis especially the one sourced from iNOS to a good extent prevents the harmful effects of aluminium in the brain thereby preventing the resultant cognitive and behavioral impairment. Al accumulation in the brain can impair the neuronal glutamate-NO-cGMP pathway which may contribute to neurological derangements.[15]

Alpha amyrin is a potent phytocompound of the class of pentacyclic triterpenes. Despite of its potential, it exhibits variable GI absorption and poor BBB permeability. The compound has been shown to have antidepressant and anxiolytic activity. A nano preparation of the drug administered through a suitable route that ensures the proper drug uptake into the brain would resolve the issue associated. A chitosan-incorporated nano-emulsion of the drug alpha amyrin which when administered through the intranasal route would be carried by the olfactory and trigeminal nerves supplying the nasal mucosa directly to the brain. Chitosan is a natural polymer well known for its BBB permeability-enhancing property. The nano-size of the formulation, chitosan incorporation, and intranasal route of administration of the drug alpha amyrin would ensure protection against neuronal inflammation, and degeneration and would correct deranged neurotransmitter levels brought about by aluminium chloride-induced neuro-toxicity.[16]

In silico approaches helps to identify the interactions between a drug or a ligand with the protein or enzyme involved in its target site of action without the moiety being tested on animals. This helps to speed up the process of drug discovery and lead optimization at the same time minimizes the costs, the chance of failures, and the time incurred. Computational tools and molecular docking provide a two-dimensional or three-dimensional form of the drug, which can then when rotated be viewed from any angle showing various sites to which different enzymes and proteins can bind and the extent of interaction, orientation, and affinity between the catalytic binding site of the protein target and lead/drug can be effectively determined based on docking scores.

The purpose of the study is to determine the neuroprotective role of alpha amyrin nano-emulsion administered intranasally against neuroinflammation, neuronal damage, and excitotoxicity via an *in silico* and *in vivo* approach.

MATERIALS AND METHODS

In silico molecular docking study of alpha amyrin

In silico approach involving molecular docking was performed to identify the interactions and orientations of the lead moiety alpha amyrin with the targets involved in the neuroinflammatory process. The binding site as well as the interacting facet of enzymes were identified. Docking studies were performed by AutoDock 4.2. Molecular docking aided *a* better understanding of the interaction between alpha amyrin and inflammatory mediators like TNF-α, IL-6, NF-KB, nNOS and iNOS.

Formulation of Chitosan decorated nano-emulsion of alpha amyrin

Chemicals and Apparatus

Alpha amyrin (98% pure), glacial acetic acid, de-ionized water, Chitosan (ICAR), Tween 80, PEG-400, sesame oil.

The preparation and optimization of the alpha amyrin chitosan Nanoemulsion (NE) formulation were carried out using a spontaneous emulsification technique at a temperature of 25°C. Both the oil phase and aqueous phase were prepared separately. The organic phase was obtained by combining the drug, sesame oil, and polyethylene glycol under continuous stirring. The aqueous phase consisted of a chitosan solution and Tween 80. To prepare the nanoemulsion, the organic phase was slowly added to the aqueous phase while continuously stirring. The resulting mixture was further stirred to ensure proper emulsification. Chitosan-decorated nanoemulsions were prepared by adding 20 mg of chitosan (low molecular weight, approximately 50 kDa) to 100 mL of 1% glacial acetic acid. The polymer solution was stirred to optimize its viscosity.

To this chitosan solution, 2.5 mL of Tween 80 was added and blended well for 20 min. The oil phase, the drug was mixed with 10 mL sesame oil and 5% Polyethylene glycol, was stirred at high speed for 1 hr. The oil phase was then added dropwise to the aqueous phase and agitated for 60 min at room temperature with continuous stirring at 2000 rpm using a Kinematica Polytron™ PT2100.

The resulting mixture was further homogenized at high speed (4000 rpm) for 2 min to ensure formulation homogeneity. To achieve further size reduction, the nanoformulation was passed through a 0.02-micron syringe filter.[17-20]

The intranasal route was selected for administering alpha amyrin nanoemulsion as it was identified as non invasive,easy to administer and best suited to ensure drug efficacy even at small doses. It is a promising approach for targeting the brain where the drug directly gets carried to the brain via olfactory pathways without any first-pass metabolism.[21]

Chitosan, a natural polymer enhances absorption of the drug when administered intranasally as it enhances its permeability and transfer to the brain through olfactory and trigeminal nerves.[22] Sesame oil is considered to enhance the absorption of drugs across the nasal epithelium. Polyethylene glycol and tween were used as surfactants.[23]

The resultant nano-emulsion is evaluated and characterized for its particle size zeta potential, surface morphology, viscosity, thermodynamic stability, percentage drug content, and TEM analysis.

In silico prediction of the anti-inflammatory role of alpha amyrin in neuronal inflammation

The NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) transcription factor family plays a crucial role as a versatile regulator of various cellular signaling pathways, orchestrating inflammatory responses in cells exposed to a wide range of stimuli. Among the key factors involved in these responses are the cytokines TNF-alpha and IL-6, which are primarily responsible for initiating the production of acute-phase proteins like CRP and promoting an inflammatory state. NF-κB governs the expression of nearly 500 different genes, including enzymes such as Cyclooxygenase (COX)-2, 5-Lipoxygenase (LOX), and inducible NO Synthase (iNOS), as well as cytokines like Interleukin (IL)-1, IL-6, IL-8, chemokines, and Tumor Necrosis Factor (TNF), and adhesion molecules.

Activation of NF-κB in microglial cells plays a central role in triggering the release of reactive oxygen species and proinflammatory cytokines, including IL-1β, interferon-γ, and TNF-α. This activation can lead to secondary neurotoxicity, contributing to the progression of neuroinflammation. NF-κB acts as a crucial mediator in various cellular signaling pathways involved in inflammation. The cytokines TNF-alpha and IL-6 drive the induction of acute-phase proteins and promote an inflammatory state. NF-κB regulates the expression of numerous genes, including enzymes, cytokines, and adhesion molecules. Activation of NF-κB in microglial cells triggers the release of reactive oxygen species and proinflammatory cytokines, which can cause secondary neurotoxicity in the context of neuroinflammation.[24]

In certain models of Alzheimer's Disease (AD), the absence of Inducible Nitric Oxide Synthase (iNOS) leads to significant improvements. iNOS deficiency results in reduced beta-amyloid plaques and phosphorylated tau protein, as well as increased survival rates. Nitric Oxide (NO) production within the central nervous system during inflammation contributes to neuronal death through the release of glutamate and subsequent excitotoxic cell death. Inhibiting NO production rescues neurons from death, as demonstrated both in laboratory studies and animal models. Excessive NO production in the context of neuroinflammation is now recognized as a crucial pathological factor in diseases such as

Alzheimer's disease. In the iNOS-deficient mouse model, reduced levels of beta-amyloid, fewer plaques, diminished phosphorylated tau protein and improved survival are observed compared to mice expressing iNOS.[25] In the above context, for docking studies, specific targets like NF-kB, TNF-α, IL-6, iNOS, and nNOS were selected to determine how far the drug alpha amyrin interacts with them.For the purpose of docking, Autodock 4.2 was used.

To ensure accuracy and reliability in docking simulations validation steps were taken. to assess the performance and predictive capabilities of the docking method used. They include Benchmarking against experimental data: Docking results were compared with experimental data, where the drug alpha amyrin and its impact on brain levels of inflammatory markers through docking and *in vivo* methods were compared.

Virtual screening was performed to determine the specific target which involves docking of the compound against the target protein to identify potential binders.

Pilot study

The drug alpha amyrin is known to be administered orally for its pharmacological effect. But in the present study, oral administration did not give good results, also computational tools and certain literature confirm that alpha amyrin shows erratic GI absorption and poor BBB permeability. So nasal route that surpasses GI transit, first-pass metabolism, and protein binding is opted. Nasal administration ensures the direct transport of drugs from the nasal epithelium direct to the brain. The poor solubility of the drug in water and solvents made us adopt a nanoemulsion form of the drug. Supporting literature for nasal doses of alpha amyrin was not available for which a pilot study was performed to select a suitable dose.

Different doses of alpha amyrin nanoemulsion such as 2.5 mg/mL, 5 mg/mL,10 mg/mL, and 20 mg/mL prepared by means of the spontaneous emulsification method. Five groups of rats consisting of 3 animals per group were administered with four different doses of alpha amyrin nanoemulsion such as 2.5 mg/mL, 5 mg/mL,10 mg/mL, and 20 mg/mL at a volume of 25 µL[26] in each nostril followed by $AlCl_3$ administration orally after an hour (100 mg/mL) for 42 days. One group served as Sham which received 25 µL of distilled water in both nares and one group acted as the control group receiving $AlCL_3$ (100 mg/mL orally). The grouping was as follows:

Group I: Normal group administered with normal distilled water (25 µL in each nostril),

Group II: $AlCl_3$ group (induced neuro-toxicity) (100 mg/kg p.o $AlCl_3$ p.o.),[27]

Group III: Alpha amyrin 2.5 mg/mL alpha amyrin nanoemulsion administered IN at a dose of 25µL to both nares (dose equivalent to 0.05 mg/day))+$AlCl_3$ (100 mg/kg p.o),

Group IV: Alpha amyrin (5 mg/mL alpha amyrin nanoemulsion (IN) administered into both nares (25 µL in each nostril) (dose equivalent to 0.1 mg/day) + $AlCl_3$ (100 mg/kg p.o),

Group V: Alpha amyrin (10 mg/mL alpha amyrin nanoemulsion(IN) administered into both nares (25 µL in each nostril) (dose equivalent to 0.2 mg/day) + $AlCl_3$ (100 mg/kg p.o).

Group VI: Alpha amyrin (20 mg/mL alpha amyrin nanoemulsion (IN) administered into both nares (25 µL in each nostril) (dose equivalent to 0.4 mg/day) + $AlCl_3$ (100 mg/kg p.o).

Administered 50 µL of nano-emulsion by means of a micropipette, 5 µL per nostril each time, which was alternated between nostrils every 2-3 min, and animals were treated for 42 days.[28] The animals were tested using an elevated plus maze for their neurobehavioral changes on day 21 and day 42. The dose and route,[29,30] of administration of isolated alpha amyrin were selected based on a pilot study and earlier literature.

Experimental animals

32 male Wistar albino rats weighing 200-250g were procured from Krupanidhi College of Pharmacy, Bangalore, India. They were housed and acclimatized in a well-ventilated animal house. Laboratory conditions were maintained for 10 days prior to the experiment in a controlled temperature (25±4°C) and relative humidity (50-60)% with 12 hr light and dark cycle with food and water ad libitum as per the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional Ethical Committee approved the experiment protocol by the number KCP/IAEC/PCOL/61/2020, Induction of neuro-toxicity with Aluminium chloride and evaluation of neuroprotective activity in animals.

To carry out the present investigation, for the experimental design, Male Wistar albino rats (n=8) were grouped as follows:

Group I: Normal group with normal distilled water,

Group II: $AlCl_3$ group (induced neuro-toxicity) (100 mg/mL p.o $AlCl_3$ p.o.),

Group III: Alpha amyrin (10 mg/mL IN)+$AlCl_3$ (100 mg/mL p.o),

Group IV: Alpha amyrin (20 mg/mL IN)+$AlCl_3$ (100 mg/mL p.o), The dose and route of administration of isolated alpha amyrin were selected based on earlier scientific literature and based on pilot studies.

Preparation of Drug for administration

The chitosan decorated alpha amyrin nano-emulsion was administered intranasally with a micropipette, inserted approximately 5 mm at a volume of 50 µL with the animals in a supine position into the right nostril and left olfactory bulb remains as control. Animals were tested for the parameters on days 21 and 42. At the end of the 42 days study, animals were

sacrificed and the brain was dissected out and washed with ice-cold isotonic saline maintained at -80°C. Two animals from each group were maintained separately for histopathological studies.

The brain hippocampus and cortex areas were separated and washed in ice-cold saline and were homogenized using a homogenizer with 10 parts of ice-cold phosphate buffer solution (0.1M) maintained at pH 7.4. The homogenate was then centrifuged for 20 min at 4000 rpm and the supernatant was collected to perform biochemical estimations.

Estimation of SOD

The measurement of Superoxide Dismutase (SOD) activity involved the detection of superoxide radicals (O_2-) through their oxidation by hydroxylamine hydrochloride, resulting in the production of nitrite. The level of nitrite was quantified using colorimetry at a wavelength of 560 nm. Additionally, the auto-oxidation of hydroxylamine at pH 10.2 also generates superoxide radicals. In the presence of EDTA, the reduction of Nitro Blue Tetrazolium (NBT) occurs, leading to the production of nitrite, which was measured using colorimetry.

The units of SOD were defined as the enzyme quantity required to inhibit the reduction of Nitro Blue Tetrazolium (NBT) by 50%. The specific activity of SOD was expressed as units per milligram (mg) of protein. A parallel control sample without tissue homogenate underwent the same procedure to account for any non-specific reactions. The SOD activity was reported as units per milligram of protein (units/mg).[31,32]

Estimation of Catalase

Catalase activity can be determined using a spectrophotometric method. The assay involves measuring the breakdown of hydrogen peroxide (H_2O_2) by catalase, resulting in the production of water and oxygen. The rate of this reaction is assessed by monitoring the decrease in absorbance at a specific wavelength, typically 240 nm, as H_2O_2 is consumed. This change in absorbance over time serves as a measure of catalase activity and was measured by colorimetry.

To perform the assay, a reaction mixture is prepared, containing the sample (containing catalase), hydrogen peroxide, and an appropriate buffer. The mixture is then incubated at a specific temperature, typically 25°C, for a defined period of time. Following incubation, the reaction is halted by adding a stop solution, such as sodium azide. The absorbance of the reaction mixture is measured at 240 nm, and the resulting change in absorbance is utilized to calculate catalase activity. Catalase activity is expressed in units, where one unit is defined as the amount of enzyme that catalyzes the breakdown of 1 µmol of H_2O_2 per minute under the given assay conditions.[33]

Brain levels of Acetylcholinesterase (AChE)[34]

Aluminium deranges the cholinergic system causing shoot up of brain AChE levels. The AChE activity in rat brains was determined following the method prescribed by Ellman *et al.* with suitable modifications. The procedure made use of electric eel acetylcholinesterase (*Native electrophorus electricus acetylcholine esterase,* creative enzymes, NATE-0018) and Acetyl Thiocholine Iodide (ATCI) served as the substrate and 5, 5-dithiobis (2- nitrobenzoic) acid (DTNB), an -SH reagent was utilized to estimate AChE activity. The reaction mixture consisted of 150 µL of 0.1 M sodium phosphate buffer (pH 8.0), 10 µL of supernatant brain homogenate, and 20 µL enzyme solution (0.09 units/mL). All the above were mixed and incubated for 15 min at 25°C. About 10 µL of DTNB (10 mM) was added to the mixture and initiated the reaction process by adding the substrate (10 µL of ATCI, 14 mM solution). The process involves hydrolysis of the ATCI resulting in the formation of the colored product 5- thio-2-nitrobenzoate anion due to the reaction of DTNB and thiocholine, which is released by the hydrolysis of the enzyme. This is measured after 10 min at 410 nm using the *spectrophotometric method*. Physostigmine dissolved in ethanol (AchE inhibitor), was used as a toxic control. Percentage inhibition of acetylcholinesterase was calculated using the following formula.

$$\text{Percentage inhibition} = \frac{\text{Control OD-Test OD}}{\text{Control OD}} \times 100$$

Brain levels of Glutamate[35]

Rat brain was homogenized with 2 parts by weight of perchloric acid and centrifuged for 10 min at 3,000 rpm. 3 mL of supernatant fluid was adjusted to pH 9 with 1 mL phosphate solution and rested for 10 min in an ice bath followed by filtering it through fluted filter paper The absorbance was measured at 340 nm. A blank reading at 340 nm was measured using UV spectrophotometric method.

Estimation of inflammatory markers[36-38]

The quantification of IL-6 and TNF-α present in the hippocampus and prefrontal cortex was determined using ELISA assays, and the instructions supplied by the manufacturer were followed (*Millipore*). The results are expressed in ng of cytokine per mL of brain tissue homogenate.

Histopathological Studies

Performed histopathology study with brain tissues. The brain dissected out from the animal was immersed and washed in ice-cold saline followed by immersing the specimen in 10% formalin. Transverse sections of the hippocampus and cortex areas were sliced out from brain tissue and fixed in paraffin blocks using a microtome. *Hematoxylin* (to stain nucleus) *and eosin* (to stain cytoplasm) *(H&E)* was used to stain the thin sections which were then examined under a digital microscope at a magnification

of 50 X,100 X, and 400 X. Six slides per group were evaluated for the cortical, hippocampal, and pyramidal regions. The evaluation was done based on the number of intact neuronal cells observed.

Statistical methods

Statistical significance of all the results were tested by comparing treatment groups with the respective toxic control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as Mean±SD.

RESULTS

The alpha amyrin nano-emulsion was found to be thermodynamically and physically stable after undergoing heating and cooling cycles and stress conditions. The globule size is determined through Horiba scientific 75.9±5.4nm, zeta potential 25.6 mV, PDI-0.39, and pH-4.9 with a percentage drug content of 79%. The average size distribution of prepared nano-emulsion was in the range of 50-100 nm with a PDI between 0.3 and 0.9 which suggests the formation of nano-sized formulations. The TEM analysis image of alpha amyrin is shown below (Figure 1).

In silico prediction of the anti-inflammatory role of alpha amyrin in neuronal inflammation

The molecular docking of alpha amyrin with the crystalline structure of IL-6 (4CNI) shows a docking score of -7.67 kcal/mol. The compound effectively interacts with the catalytic binding sites of IL-6 causing its inhibition (Figure 2).

Docking of alpha amyrin with the crystalline structure of NF-KB shows a docking score of -6.7. The compound interacts with the catalytic sites of NFKB(1NFI) a key mediator of oxidative stress and inflammation in the brain in case of aluminum-induced neuro-toxicity and thereby inhibits its functioning. NF-κB prompts the expression of pro-inflammatory mediators, cytokines, and chemokines and engages in the inflammatory process. In addition, NF-κB plays a critical role in regulating the survival, activation, and differentiation of innate immune cells and inflammatory T cells (Figure 3).

Alpha amyrin docked with TNF-α (1TNF) showed a docking score of -10.2, indicating that alpha amyrin interacts with TNF-α with high affinity to inhibit its action (Figure 4).

The results of docking show that alpha amyrin effectively interacts with IL-6, TNF-α, and NFKB and thereby inhibits them reducing the inflammatory cascades resulting from aluminum-induced damage. It attenuates BDNF too.

Alpha amyrin docks nNOS with a docking score of -11.3. (Figure 5) and docks with iNOS with a docking score of -9.7 (Figure 6).

Neuroprotective studies

Pilot study

In the study, four different doses of alpha amyrin nanoemulsion (2.5 mg/mL, 5mg/mL, 10 mg/mL, and 20 mg/mL) were administered at a volume of 25 µL in each nostril to experimental groups of animals followed by $AlCl_3$ administration along with a sham group. The animals were tested for their initial, first, and second transfer latency in an elevated plus maze test and the results are given in Figures 7-9. The findings implicate that the treatment was most effective at a dose of 10 mg/mL and 20 mg/mL as the transfer latency was reduced evidently with the aforementioned doses. For their effective results in the elevated plus maze test, the doses 10 mg/mL and 20 mg/mL were selected for the treatments.

In vivo studies on rat brain

Estimation of brain levels of SOD and Catalase

The rat brain levels of SOD (Table 1), and Catalase (Table 2) that diminished with aluminium administration were regulated and elevated with alpha amyrin nanoemulsion treatment, especially at a dose of 20 mg/mL.

Estimation of brain levels of AChE

The acetylcholine esterase levels in rat brains were found to increase with aluminium exposure whereas administration of alpha amyrin nano-emulsion normalized the AchE levels in a dose-dependent manner (Table 3).

Estimation of brain levels of glutamate

The glutamate level in the brain was found to decrease in a dose-dependent manner with the Alpha amyrin NE administration at a dose of 20mg/mL (10.01±0.03) when compared to the induced group (15.98±0.01). Even the decreased value was near the normal level as that recorded in the normal group of animals (5.61+0.07) (Table 4).

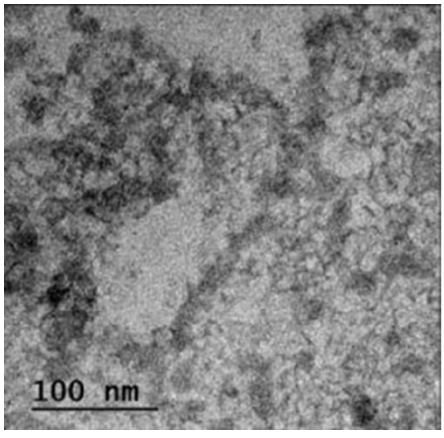


Figure 1: TEM analysis image.

Estimation of brain levels of inflammatory markers

Estimation of brain levels of IL-6

The brain levels of IL-6 increased in the aluminum-administered group compared to the normal group indicative of the inflammatory prototype. Alpha amyrin nano-emulsion lowered the IL-6 levels in rat brains (Table 5).

Estimation of brain levels of TNF-α

Elevated brain levels of TNF-α was observed in aluminium induced group whereas alpha amyrin nano-emulsion administration lowered the levels of TNF-α in rat brain (Table 6).

The above results indicate the protective role of alpha amyrin nano-emulsion against neuro-inflammation induced by aluminium and its role in correcting aberrated levels of acetylcholine esterase and glutamate in rat brains and its protective effect against combating neuronal inflammation by regulating levels of IL-6 and TNF-α in the brain.

One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as Mean±SD. The significance level is set at 0.05. If the *p*-value is equal to or smaller than 0.05, it rejects the null hypothesis. This decision leads to the conclusion that not all population means are equal, suggesting the presence of a significant difference among the groups or variables being compared.

Analysis of variance helps determining whether significant differences exist among the means of three or more groups. When performing ANOVA, if the null hypothesis (H0) is rejected, it indicates that there are overall differences among the groups. ANOVA reveals the presence of significant differences among the groups collectively, it does not directly indicate the specific group pairs that differ from each other. To determine the specific group differences, Dunnett's test, a post hoc test, which is conducted after a significant one-way Analysis Of Variance (ANOVA), to identify significant differences among treatment means compared to a control group mean. It is useful when conducting multiple comparisons of treatment means against a single control group mean and helps determine which specific differences are statistically significant in a comprehensive manner among various groups or combinations of groups for which it is employed in the current study.

Table 1: Estimation of brain levels of SOD.

Sl. No.	Treatment group	Brain levels of SOD U/mg protein
1	Normal group	1.137±0.059
2	$AlCl_3$ group	0.347±0.049[a]
3	Alpha amyrin NE 10 mg/mL	0.883±0.037[b]
4	Alpha amyrin NE 20 mg/mL	1.03±0.021[c]

Statistical significance of SOD levels was assessed by comparing treatment groups with the respective toxic control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6), and [a] p<0.001 when compared to the normal group. [b, c]p<0.001 and when compared to the toxic control group.

Table 2: Estimation of brain levels of Catalase.

Sl. No.	Treatment group	Brain levels of Catalase U/mL
1	Normal group	7.15±0.24
2	$AlCl_3$ group	4.01±0.184[a]
3	Alpha amyrin NE 10mg/mL	4.47±0.21[b]
4	Alpha amyrin NE 20mg/mL	5.095±0.21[c]

Statistical significance of Glutamate levels was assessed by comparing treatment groups with the respective toxic control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6), and [a] p<0.001 when compared to the normal group. [b, c]p<0.001 and when compared to the toxic control group.

Table 3: Estimation of brain levels of AChE.

Sl. No.	Treatment group	Brain levels of AChE (µmol/min/g)
1	Normal group	5.61±0.09
2	$AlCl_3$ group	15.18±0.1[a]
3	Alpha amyrin NE 10 mg/mL	11.23±0.2[b]
4	Alpha amyrin NE 20 mg/mL	10.01±0.05[c]

Statistical significance of AChE levels was assessed by comparing treatment groups with the respective toxic control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6), and [a] p<0.001 when compared to the normal group. [b, c]p<0.001 and when compared to the toxic control group.

HISTOPATHOLOGICAL STUDIES

Histopathology of the brain-normal group: Figure 10 shows the histopathology of different brain regions in normal group animals. Slides 1 and 2 with the *c*ortical region showing *g*lial cells and neuronal cells with normal morphology (100X), and neuronal cells with normal morphology (X 400) Slide no: 3,4: Hippocampus region showing normal morphology (X 100) and (X 400). Slides no:5 and 6 show the *p*yramidal region showing Purkinje Cells (PC) normal (100 X) morphology (X 400).

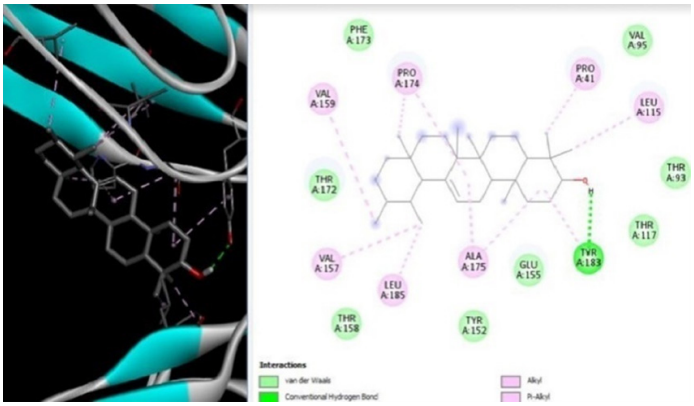


Figure 2: Docking image of alpha amyrin with IL-6.

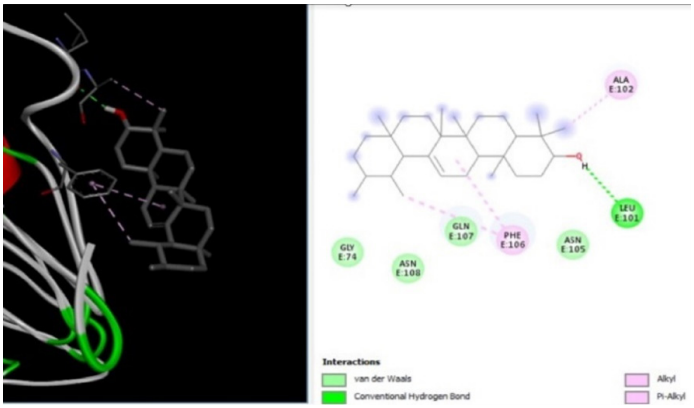


Figure 3: Docking image of alpha amyrin with NF-KB.

Table 4: Estimation of brain levels of Glutamate.

Sl. No.	Treatment group	Brain levels of Glutamate (nmol/min/g)
1	Normal group	0.115±0.014
2	$AlCl_3$ group	0.289±0.022[a]
3	Alpha amyrin NE 10 mg/mL	0.222±0.016[b]
4	Alpha amyrin NE 20 mg/mL	0.142±0.010[c]

Statistical significance of glutamate levels was determined by comparing treatment groups with the respective toxic control group by employing One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6), and [a] p<0.001 when compared to the normal group. [b,c]p<0.001 and when compared to the toxic control group.

Table 5: Estimation of brain levels of inflammatory markers IL-6.

Sl. No	Treatment group	Brain levels of IL-6 (pg/mL)
1	Normal group	281.0± 4.1
2	$AlCl_3$ group	682.0±1.9[a]
3	Alpha amyrin NE 10 mg/mL	608.0±2.5[b]
4	Alpha amyrin NE 20 mg/mL	561.0±2.2[c]

Statistical significance of IL-6 results was determined by comparing treatment groups with the respective toxic control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6), and [a] p<0.001 when compared to the normal group. [b,c]p<0.01 and when compared to the toxic control group.

Table 6: Estimation of brain levels of inflammatory markers TNF-α.

Sl. No	Treatment group	Brain levels of TNF-α (pg/mL)
1	Normal group	50.08±0.4
2	$AlCl_3$ group	113.43±0.33[a]
3	Alpha amyrin NE 10 mg/mL	67.56±0.04[b]
4	Alpha amyrin NE 20 mg/mL	48.96±0.7[c]

Statistical significance of TNF-α results was tested by comparing treatment groups with the respective toxic control group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6), and [a] p<0.001 when compared to the normal group. [b,c]p<0.001 and when compared to the toxic control group.

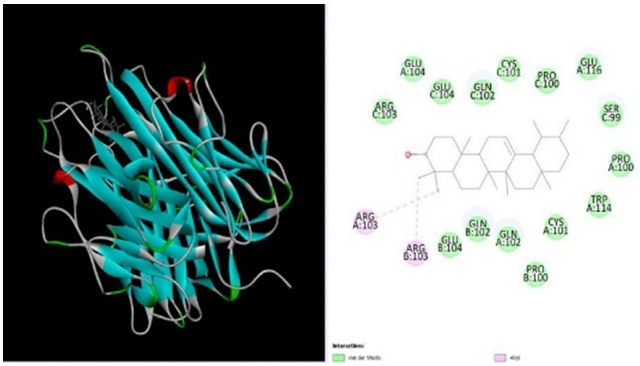


Figure 4: Docking image of alpha amyrin with TNF-α.

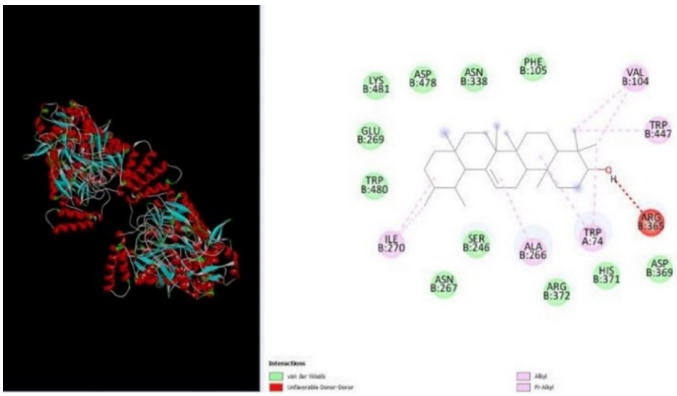


Figure 5: Docking image of alpha amyrin with iNOS.

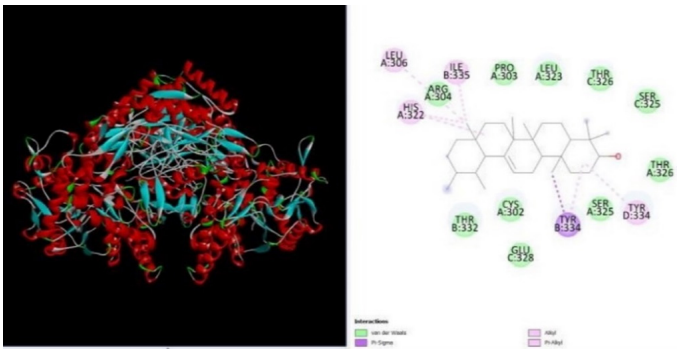


Figure 6: Docking image of alpha amyrin with nNOS.

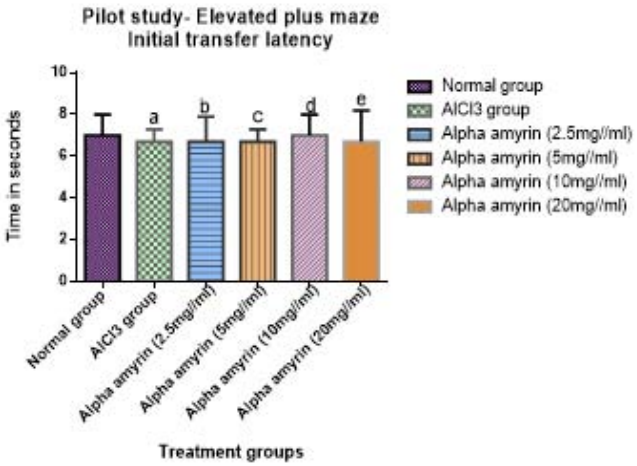


Figure 7: Initial transfer latency in elevated plus maze test.

Statistical significance of initial transfer latency of rats in elevated plus maze test was assessed by comparing treatment groups with the normal group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6). $^{b,c,d,e}p<0.05$ when compared to the normal group.

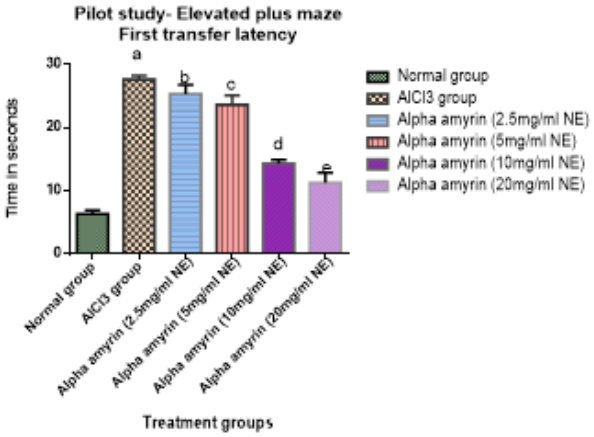


Figure 8: First transfer latency in elevated plus maze test.

Statistical significance of first transfer latency was assessed by comparing treatment groups with the respective $AlCl_3$ group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6), and $^{b}p<0.001$ when compared to the normal group. $^{b,c,d,e}p<0.001$ and when compared to the $AlCl_3$ induced toxic control group.

Histopathology of the brain-Aluminium induced group: (*Toxic* control group) Figure 11 shows the histopathology of different brain regions in the toxic control group) Slide no: 7, 8 shows the cortical region showing gliosis and Pyknotic nuclei (Pn) fatty vacuolation. Slide no: 9, 10 shows the hippocampus showing reduced neuronal populations.

The presence of blood vessel congestion, apoptosis gliosis, and degenerative fatty vacuolations was moderate. Slide no: 11, 12 shows the pyramidal region showing fatty vacuolations and apoptosis.

Histopathology of the brain-Alpha amyrin 10 mg/mL treated group: Figure 12 shows the histopathology of different brain regions in the Alpha amyrin 10 mg/mL treatment group. Slide no: 13, 14 with the cortical region showing gliosis- focal: 1+ (X50) (both neuronal cells and glial cells showing normal morphology). Slide no: 15, 16 with the hippocampus showing near normal

morphology (X 50) and showing normal neuronal cells (X 100). Slide no: 17, 18: Pyramidal region showing normal morphology (X 50) and showing near normal Purkinje cells (PC)-(X100).

Histopathology of the brain-Alpha amyrin 20 mg/mL treated group: Figure 13 shows the histopathology of different brain regions in the Alpha amyrin 20 mg/mL treatment group. Slides 19 and 20 show the Cortical region with normal morphology-(X50) Cortical region showing gliosis-focal: 1+(Neuronal cells normal morphology-NAD+) (X100). Slides 21 and 22 with hippocampus showing near normal morphology (X 50) 22 and showing normal neuronal cells (X 100). Slides 23 and 24 with the pyramidal region showing near normal morphology (50 X) and showing near normal Purkinje cells (100 X).

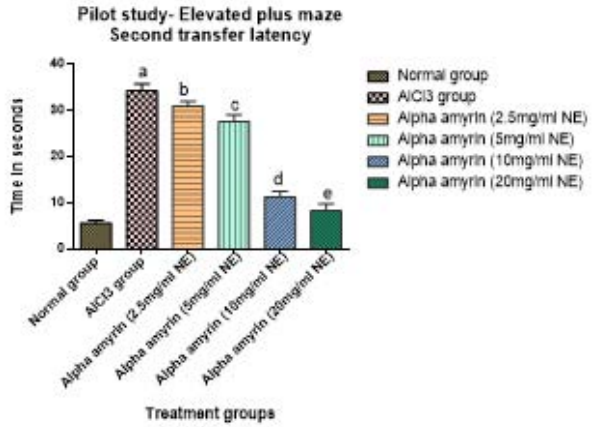


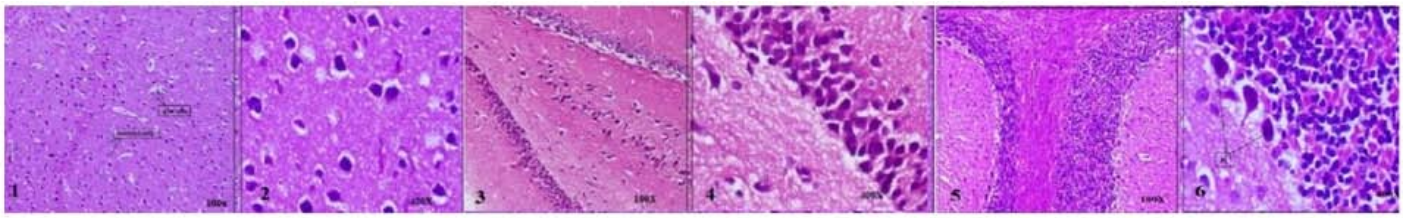
Figure 9: Second transfer latency in elevated plus maze test.

Statistical significance of second transfer latency was assessed by comparing treatment groups with the respective $AlCl_3$ group by means of One-way ANOVA ordinary measures followed by Dunnett's comparison test where data are expressed as mean±SD (n=6), and $^{b}p<0.001$ when compared to the normal group. $^{b,c,d,e}p<0.001$ and when compared to the $AlCl_3$ induced toxic control group.

DISCUSSION

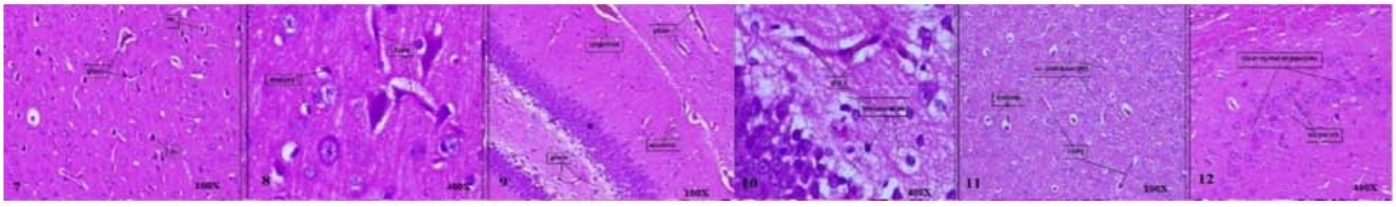
Alpha amyrin a pentacyclic triterpene of the ursane group was formulated into a nano-emulsion targeting the brain and administered via the intranasal route to animals. Docking studies were performed to determine the possible interaction of the drug with various mediators of neuronal inflammation. This was followed by *in vivo* studies performed on animals treated with alpha amyrin nanoemulsion administered for a period of 42 days.

Intranasal administration holds promise as a potential option for rescuing compounds that face challenges in achieving adequate distribution to the brain through the systemic circulation. Alpha amyrin administered orally showed poor BBB permeability and erratic GI absorption for which an alternate route focusing brain was selected. A chitosan-decorated nano-emulsion form of the drug with sesame oil as the base helped accentuate the drug's permeability to the brain when administered intranasally. In another study investigating the intranasal administration of low-permeable compounds, it was noted that in rats, a majority



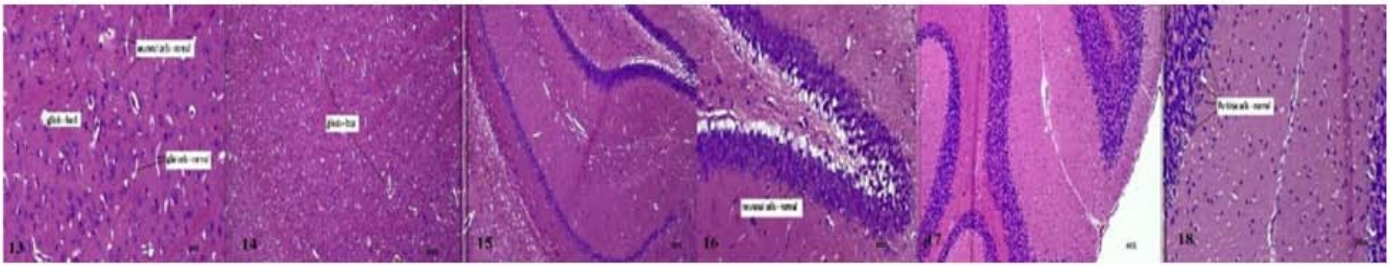
Slides 1,2- Cortical region showing glial cells and neuronal cells normal morphology and neuronal cell normal morphology(X100) and (X400)	Slides 3,4- Hippocampus region showing normal morphology(X100) and (X400)	Slides 5,6-Pyramidal region showing Purkinje cells (PC) normal morphology (X100) and (X400)

Figure 10: Histopathology of different brain regions in the normal group.



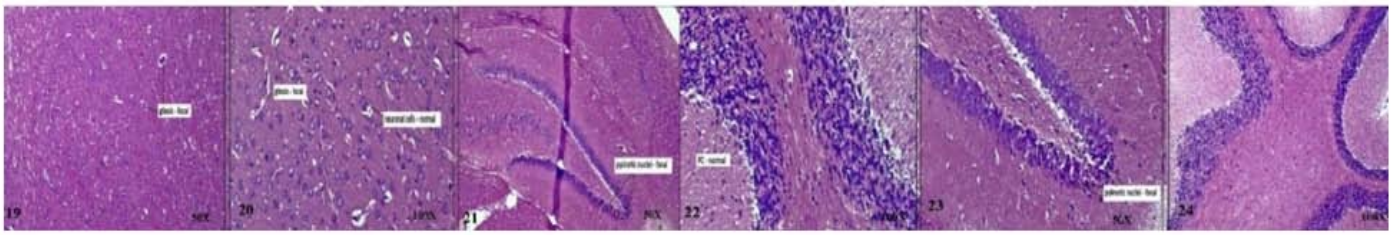
Slides 7,8- Cortical region showing gliosis & pyknotic nuclei (Pn) showing fatty degenerative vacuolations-moderate (X100) and (X400)	Slides 9,10- Hippocampus region showing blood vessel congestion, apoptosis, gliosis, hippocampus showing reduced neuronal cell populations (X100) and (X400)	Slides 11,12-Pyramidal region showing vacuolation and apoptosis moderate (X100) and (X400)

Figure 11: Histopathology of different brain regions in the toxic control group.



Slides 13,14- Cortical region showing gliosis focal (50X) Neuronal cells and glial cells showing normal morphology (X100)	Slides 15,16- Hippocampus region showing normal morphology (X50) and showing normal neuronal cells (X100)	Slides 17,18--Pyramidal region showing normal morphology (X50) showing near normal (PC) –Purkinje cells (PC) (X100)

Figure 12: Histopathology of different brain regions in the Alpha amyrin treatment group (10 mg/mL).



Slides 19,20- Cortical region showing normal morphology, gliosis focal (50X)	Slides 21,22- Hippocampus region showing normal morphology (X50) and showing normal neuronal cells (X100)	Slides 23,24- Pyramidal region showing normal morphology (X50) showing Purkinje cells (PC) with near normal morphology (X100)

Figure 13: Histopathology of different brain regions in the Alpha amyrin treatment group (20 mg/mL).

of the low-permeable compounds exhibited a significant increase in the brain/plasma concentration ratio (Kp) following intranasal administration, despite their susceptibility to efflux drug transporters. Conversely, this observation was not made with high-permeable compounds. The permeability of a compound plays a crucial role in determining the increase in Kp through intranasal administration. This route of delivery proves to be particularly advantageous for low-permeable compounds as it enhances their transportation to the brain in rodents. The intranasal route consists of two distinct pathways: the intracellular pathway and the extracellular pathway. The intracellular pathway encompasses processes such as passive diffusion, adsorptive endocytosis, and receptor-mediated endocytosis. On the other hand, extracellular transport involves multiple pathways, including bulk flow. The above two aids the transport of drug across the BBB to the brain.[39]

In the process of plant biosynthesis, α-amyrin serves as the precursor for the production of ursolic acid. To enhance the bioavailability of drugs, researchers utilize novel drug delivery systems, such as microspheres or nanoparticle-based systems. In the context of ursolic acid, chitosan nanoparticles loaded with the compound have been found to exhibit strong anti-angiogenic effects in tumors.[10,40]

Chitosan possesses polycationic amino groups that provide it with the ability to exhibit mucoadhesive properties. This characteristic enables chitosan to establish ionic interactions with anions present on the cell surface or mucous membrane. As a result, chitosan has an increased residence time at the target site, leading to enhanced absorption through the membrane. The chitosan nanoformulation loaded with the drug facilitates the transport of the drug across tight junctions, enabling its accessibility to the astrocyte endfeet that is connected to the cerebral blood vessels. Consequently, this nanoformulation enables the drug to reach the brain and become available within its tissues.[41]

Prior to animal study, docking studies were performed with the lead alpha amyrin to determine its possible role in neuro-protection and to determine the feasibility of the drug to be used for the neuroprotective study. AutoDock 4.2 was used for performing in silico docking studies for alpha amyrin against proteins involved in neuroinflammation and neuro-degeneration in aluminum-induced neurotoxicity. (IL-6 (PDB ID:4CNI), TNF-α(1TNF), NFKB(1NFI), iNOS and nNOS). Docking studies determined binding affinity and an idea of hydrogen and covalent interactions between protein and ligand. The binding and elimination of the drug from the body depend on the number of hydrogen bonds. Alpha amyrin covalently bonded to TNF-α (1TNF) with the highest ΔG of -10.2 with a strong inhibitory effect on TNF-α. It also interacted with IL-6 with the highest

ΔG of-7.67 and with NFKB with a ΔG of -6.7 which indicates its inhibitory effect on IL-6 and NF-KB. Alpha amyrin showed a docking score of -11.3 and -9.7 with nNOS and iNOS respectively indicative of its strong inhibitory effect on nitric oxide synthase a key culprit behind neuro-toxicity. Molecular docking gave an idea about the interactions between α-amyrins with active sites of IL-6, TNF-α, NFKB, nNOS, and iNOS. Autodock gave suface details of the interaction between drug and protein targets. May require better tools to determine the exact sites of interaction between drug and the targets involved.

Post the docking studies, a 42-day treatment was performed on animals where alpha amyrin nano-emulsion was administered intranasally to aluminum-treated animals. At the end of the study, animals were sacrificed, and tested the brain levels of SOD, catalase, acetylcholine esterase, and inflammatory markers like IL-6 and TNF-α. The results convey that Aluminium causes oxidative stress and led to a fall in SOD and catalase levels, elevated brain levels of acetylcholine esterase,[42] and inflammatory markers IL-6 and TNF-α indicative of the neuro-inflammatory role of aluminium. Cholinergic dysfunction associated with a shoot in acetylcholine esterase levels accompanied by a fall in levels of acetylcholine plays a crucial role in aluminum-induced cognitive and behavioral derangement. Excitotoxicity plays a significant role in neuro-toxicity resulting from aluminum. TNF-α levels are elevated with aluminum exposure, which accentuates microglial liberation of glutamate The augmentation of excitotoxicity by immune mediators like IL-6, and TNF-α occurs through interaction between the cytokine receptors and glutamate receptors resulting in excitotoxicity-mediated neuronal damage. Glutamate on the other hand cause excitotoxicity mediated damage on neurons by mediating exaggeration of the excitatory effect.[43]

Treatment with alpha amyrin nano-emulsion brought about a dose-dependent reduction in Acetylcholine esterase and glutamate levels in rat brains. Glutamate is a component involved in the excitotoxic process in the brain. AA NE lowered the excitotoxic damage induced by aluminium and at the same time brought about a reduction in the acetylcholine esterase-mediated alterations in the brain. Acetylcholine esterase is a neurotransmitter linked to neurodegenerative disorders like alzheimers as it deranges normal levels of acetyl choline. Alpha amyrin by downregulating levels of glutamate and AChE suggests the neuroprotective role of alpha amyrin in lowering excitotoxicity, neuronal apoptosis and other damages caused by aluminium exposure. Also, a decline in neuro-inflammation and neuronal loss was indicated by a decline in levels of TNF-α and IL-6. We could effectively correlate the findings of *in silico* and *in vivo* results as both indicated a significant influence of the drug alpha amyrin in interacting and downregulating expression of TNF-α and IL-6.

Components of CNS such as microglia, astrocytes, and neurons express IL-6, which badly affects memory and learning, elicits neuro-degeneration and aggravates the harmful effects of other cytokines.[44] NF-kappa B triggers the transcription of the inflammatory cytokines, interleukin 6 by promoting the IL-6 gene.NF-KB in turn is activated by various triggering factors one among the dominant inducer is family of TNF family of cytokines. NF-κB and the TNF are related and the fact was established based on the findings from knockout mice wherein the animal was impotent in triggering NF-kB as the animals are embryologically manipulated causing deletion of tumor necrosis factor.[35]

The histopathological study was carried out in histopathological evaluations to evaluate the ameliorative properties of Alpha amyrin nano-emulsion at a dose of 10 mg and 20 mg/mL treated against the Aluminium Chloride ($AlCl_3$)-induced AD in animals via the suppression of oxidative stress and neuroinflammation. In Aluminium Chloride ($AlCl_3$)-induced changes in the brain there was evidence to implicate many potential factors in all the 3 regions of the rat brain, including oxidative, damage, gliosis, apoptosis, increased congestion, and Pyknotic Nuclear condensation noticed, the same induced group showed Cortical and hippocampus - severe hyperplastic vacuolar changes, pyramidal region vacuolar changes with Purkinje cells hyperplasia were observed.

In alpha amyrin, nano-emulsion administered groups, rat brains in all the 3 regions showed near normal morphology but focal gliosis and Pyknotic nuclei were evident and could be minor changes. In treated groups, a marked reduction in Aluminium Chloride ($AlCl_3$)-induced severity with the restoration of the brain histological architecture to near-normal morphology was observed. Histopathology results thereby confirm the neuroprotective role of alpha amyrin as the treatment to a good extent lowered the grade of damage, gliosis, and pyknotic changes. The hippocampus, pyramidal region, and cortex show normal morphology, neuronal cells, and Purkinje cells with a better result with alpha amyrin administration at a dose of 20mg/mL. The results convey the neuroprotective role of alpha amyrin nano-emulsion in aluminum-induced neurotoxicity.

Neuroinflammatory disorders, such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis, are characterized by chronic inflammation in the central nervous system (CNS). These conditions are associated with an imbalance in antioxidant status, neurotransmitters, increased inflammatory markers, and impaired neuronal function. From the findings of the study, drug alpha amyrin is concatenated in downregulating reactive oxygen species mediated oxidative stress, neuroinflammatory process as it evidently downregulated TNF-α, and interleukin-6. It also regulated the levels of acetylcholinesterase and glutamate which have important implications in the context of neuroinflammatory disorders and the development of novel therapeutic interventions.

The reduction in acetylcholinesterase levels suggests that alpha amyrin may have a potential role in modulating cholinergic neurotransmission. Acetylcholinesterase is responsible for breaking down acetylcholine, a crucial neurotransmitter involved in memory, learning, and cognitive functions. Inhibiting acetylcholinesterase can enhance cholinergic transmission and potentially improve cognitive deficits seen in neuroinflammatory disorders.The decrease in glutamate levels is also significant as excessive glutamate release can lead to excitotoxicity, causing neuronal damage and cell death. By lowering glutamate levels, alpha amyrin may help mitigate excitotoxicity and protect against neurodegenerative processes.

Furthermore, the reduction in TNF-α and IL-6, which are key pro-inflammatory cytokines, suggests that alpha amyrin possesses anti-inflammatory properties. Neuroinflammatory processes involving these cytokines can contribute to neuronal damage and progression of neuroinflammatory disorders. By suppressing TNF-alpha and interleukin-6, alpha amyrin may help attenuate neuroinflammation and its detrimental effects.

Overall, the study's findings highlight the potential therapeutic benefits of alpha amyrin in neuroinflammatory disorders. Its ability to modulate neurotransmitter levels, reduce inflammation markers, and potentially protect against neurodegenerative processes signifies its potential as a novel therapeutic intervention. Further research and clinical trials are necessary to explore the full therapeutic potential of alpha amyrin and its application in developing effective treatments for neuroinflammatory disorders.

The use of nanoemulsion drug delivery system demonstrates an innovative approach to enhance drug delivery to the brain. *in vivo* assessments included in this study offer a comprehensive evaluation of the formulation's impact on various molecular targets associated with neuroinflammation. Inclusion of *in silico* techniques like docking provide additional insights into the molecular interactions between the nanoemulsion components and their targets. This combination of *in vivo* and *in silico* approaches strengthens the research findings. The study utilized a rat model, which may not fully represent the complexity of human neuroinflammatory disorders also translating findings from animal models to humans requires caution and further validation in preclinical and clinical studies. Long-term studies may be required for understanding of long-term effects and the sustainability of neuroprotective outcomes.

The potential confounding factors involved in the study that may have influenced the results would be the route of administration of the drug and the formulation properties, particle size, inclusion of chitosan as the polymer and sesame oil in the formulation, nanoemulsion formulation and even the dose of the AA NE played a great role in bringing about neuroprotection in aluminium induced neurotoxicity. Future investigations should focus on mechanistic studies, long-term follow-up, and comparative studies to further validate the findings and pave the way for potential clinical applications.

CONCLUSION

NF-κB activation is an important step that prompts inflammatory gene expression that exalts anti-apoptotic pathways. The reduction in levels of TNF- alpha and IL-6 levels in rat brains indicates the downregulation of NF-κB that in turn would downregulate neuronal apoptosis in the brain. The latter was confirmed by histopathology findings. *An increase in antioxidant status* and a fall in AChE levels and glutamate levels and a decline in levels of IL-6 and TNF-α in the AA NE treatment group along with the maintenance of a good number of intact, healthy, and viable neurons as shown in histopathological studies confirms the potential of AA NE in quashing neuro-degeneration, excitotoxicity and neuronal inflammation in aluminium induced oxidative stress

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AChE: Acetylcholine esterase; **TNFα:** Tumor necrosis factor-alpha; **IL-6:** Interleukin-6; **BBB:** Blood brain barrier; **ng:** Nanogram; **µmol:** Micro mol.

SUMMARY

In silico and *in vivo* studies with alpha amyrin confirms its neuroprotective role. Intranasal administration of alpha amyrin nanoemulsion effectively lowered oxidative stress parameters, curbed inflammatory changes and corrected neurotransmitter derangements and neurodegeneration brought about by aluminum-induced oxidative stress.

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